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Progress Report

RESISTANCE OF PINES TO BARK BEETLES

Studies on Toxicity of Resins

1956

By R.H. Smith

NOT FOR PUBLICATION

CALIFORNIA FOREST AND RANGE EXPERIMENT STATION
FOREST SERVICE, U.S. DEPARTMENT OF AGRICULTURE

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SUMMARY

This is a report of the initial research to determine if and how pine materials, primarily resin, are toxic to bark beetles. The work was almost wholly confined to the fumigant toxicity of pine resins to adults of the western pine beetle. It is part of the project which is concerned with the resistance or susceptibility of pines to bark beetles.

Considerable time and effort, much of it unsuccessful, was devoted to the establishment of procedures suitable for ascertaining facts about toxicity. Difficulties were encountered and partially solved for handling fresh resin. The techniques developed, though not fully adequate, did produce results from which certain tentative conclusions can be drawn. These are as follows.

A saturated resinous atmosphere of Jeffrey pine resin is far more toxic to the western pine beetle than ponderosa pine resin which, in turn, causes a more rapid rate of mortality than an untreated atmosphere. The hybrid between these two pines is somewhat intermediate in its effect. The differences between Jeffrey, ponderosa and the hybrid may be largely caused by the vapor concentration at saturation. That is, in a confined atmosphere at saturation, there may be as much as 10 to 15 times as much resinous vapors, by weight, with Jeffrey pine than with ponderosa.

When observations were confined to the effect of a saturated atmosphere of ponderosa pine resin on the western pine beetle, the following factors were found to be of no significance.

1. Volume of the container and area of resin
2. Height of the beetle above the resin

3. Use of 24- to 32-hour-old refrigerated beetles
4. Use of refrigerated resin
5. Different trees of ponderosa pine
6. Amount of resin
7. Increasing the time of exposure beyond 24 hours
8. Absence or presence of light

On the other hand the following factors were found to have a significant effect.

1. Degree of closure of chamber
2. Temperature
3. Sex of beetle
4. Resin vs. natural resin fractions
5. Refrigeration of beetles for more than 32 hours

The reaction of the two sexes was parallel for all the factors tested. The females had a slower rate of mortality than the males under all conditions of experimentation.

Semi-confined resinous vapors did not cause the same reaction as confined ones. Jeffrey resin was non-toxic under semi-confined conditions while ponderosa still caused a significantly greater rate of mortality.

One small test indicated that Jeffrey resin was also much more toxic than ponderosa to the western pine beetle under conditions of continuous contact. However, the hybrid resin was not different from ponderosa.

The results of these tests indicate the Jeffrey pine may be resistant to the western pine beetle because of the toxicity of its resin. The position of the Jeffrey x ponderosa hybrid is not clear.

The techniques developed show promise for use for further studies.

INTRODUCTION

This report covers the research accomplished in 1956 on Line Project "Susceptibility or resistance of pines to attack," FS-2-Ill-11-Cal of the general work project, "Tree-killing bark beetles." Of the two lines of research which were given highest priority in the project analysis, 1/ the antibiotic properties of pine resins and pine

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1/ Smith, R.H. A project analysis . . . . studies of host factors which determine susceptibility or resistance of pines to bark beetle attack. Calif. Forest & Range Expt. Sta. 34 pp. typed. July 1956.

materials were the subject of practically all work. Only very sketchy work was accomplished on the second line of research, the differential response of beetles to pine materials. Within the topic of antibiosis, virtually all effort was devoted to an investigation of the fumigant toxicity of pine resins to bark beetles.

All work was done at the Institute of Forest Genetics in Placerville. Facilities, materials, and occasionally the personnel at the Institute were made available to the work.

Mr. Archie Tunnoch, who was employed as a summer research aid, assisted in the establishment of the tests and in the collection of data. Mr. Lionel Stange, student in entomology, assisted in the analysis of the data.

In his broad coverage of plant resistance to insects, Painter 2/ states that the three major areas of resistance are: (1) Non-preference (i.e. failure of the insect to make a positive response toward the plant); (2) antibiosis (i.e. adverse effect of the plant upon the insect); and (3) tolerance (i.e. ability of the plant to overcome the effects of the insect's attack). He also states that resistance studies should be concerned with the plant tissues and materials which are intimately and biologically associated with the insect.

There are repeated references or inferences in the literature to the "killing" of bark beetles by pine resins. Such remarks have been based on incidental observations without controlled analytical data as justification. Callaham 3/ suggests that resin may be toxic through both quality and quantity. He also cites the similarity between the molecular structure of some of the components of pine turpentine and known insecticides.

With these statements as a background, it was decided that pine resin was a logical material to consider for toxicity studies. It was also decided that the work at first would be confined to gross fresh resin. Work with fractionated components of resin was not considered desirable because: (1) the beetle encounters whole resin and (2) current fractionating procedure could conceivably change both the chemical and physical properties of resin components.

There are three recognized ways in which a material may be directly toxic to an insect. These are through fumigation, contact, and stomach action. When the habits of bark beetles are considered, all three modes of action appear to be possible.

2/ Painter, R.H. Insect resistance in crop plants, 520 pp. illus. 1951. New York.

3/ Callaham, R.Z. Studies of the resistance of pines to bark-beetles, season of 1952. U.S.D.A. B.F.P.Q., Forest Ins. Invest. Berkeley, Calif. 27 pp. illus. June 1953.

The testing of contact and stomach action presented many problems because of the difficulty in handling fresh resin. Certain aspects of fumigation presented fewer problems and therefore the season's effort was concentrated on fumigant toxicity studies. A report of the very limited work which was devoted to contact toxicity will be found at the end of the material on fumigant toxicity.

After a section devoted to the general procedures, the report is presented by grouping similar test. Since this does not give the chronological order of the experiments, and such chronology may be desirable, each test is given a number which does represent the order of accomplishment.

For each test there is a brief statement of purpose and conditions. The data are given in tables which include any statistical analysis. The salient points are given in a "Discussion" at the end of each test or set of tests if more than one test was devoted to the same subject. The detailed data, because of their bulk, will be held in Berkeley. The t-test values are located in the appropriate "day" columns.

FUMIGANT TOXICITY STUDIES
---GENERAL PROCEDURES---

Beetles

All beetles in all tests were western pine beetle adults, Dendroctonus brevicomis Lec., hereafter referred to as D.b. They were reared from infested ponderosa pine bark in a 6-ft. x 6-ft. x 3½-ft. walk-in cage located in the basement of the seed lab building at the Institute of Forest Genetics. Several different brood trees - all from the vicinity of the Institute - were used during the course of the work. The bark was removed from the trees while the insect was in the late larval or pupal stage. The bark was stored in a cold room at 35°F., until needed. Then it was removed and placed in the rearing cage where the daily temperature ranged from 68°F., to 78°F.

Beetles, which were usually collected twice daily at 8:00 a.m. and 6:00 p.m., were placed individually in gelatin capsules. It was usually necessary to keep them under refrigeration so that a sufficient number could be accumulated to meet the requirements of a test. The storage period varied from 12 to 48 hours. The storage temperature was about 35°F., except for the first few tests in which temperature was about 45°F.

Just before use, all beetles were equitably distributed by size and age among all the replicates of each test.

Resin

The resin was extracted from the tree using the procedure described by Callaham. [4/] A punch wound 1½ inch in diameter was made through the bark and about ½ inch into the sapwood (figure 1). A plastic cylinder was pushed into the hole and the resin collected in a 30 cc. shell vial which was attached to a hole in the lower surface of the cylinder. It was difficult to obtain resin which had not yet started to crystallize. Various procedures were tried before a suitable one was found. The most effective one was to tap the tree early in the morning and change the collecting vial at noon. By late afternoon there was usually sufficient uncrystallized resin to conduct a test. The important part of the procedure was to collect the resin which flowed after the first couple of hours of flow. The first resin which flowed seemed to crystallize more rapidly than that which flowed afterwards.

In all tests, except one which is properly noted, the same three trees, one a Pinus ponderosa Laws, one a Pinus jeffreyi Grev & Balf., and one a Pinus jeffreyi x ponderosa, [5/] were used. These will be referred to as P.p., P.j., and P.j. x p. respectively.

Assembly of test

Unless specifically noted in the details of a test, the fumigation chamber was a cork-stoppered 30 cc. test tube. The resin was apportioned with a pipette. At first the resin was placed directly in the bottom of the tube. Later it was found easier to first place the resin in a separate small resin vial.

The actual assembly was as follows (figure 2).

1. A replicate of beetles was taken from refrigeration. (The handling of the beetles was greatly facilitated by using them just out of refrigeration. They were practically inactive for the first few minutes.) Each beetle was transferred from its gelatin capsule into a fumigation cell, four such cells being bundled together.

2. The resin vial was placed in the bottom of the chamber.

4/ Callaham, R.Z. Oleoresin production in the resistance of ponderosa pine to bark beetles. Calif. Forest and Range Expt. Sta. (mimeo). May 1955, pg. 9.

5/ There is no author for this hybrid. It was first noted by N.T. Mirov in the Journal of Forestry (XXX 1, pg. 93, Jan. 1932) in "A note on Jeffrey and western yellow pine."

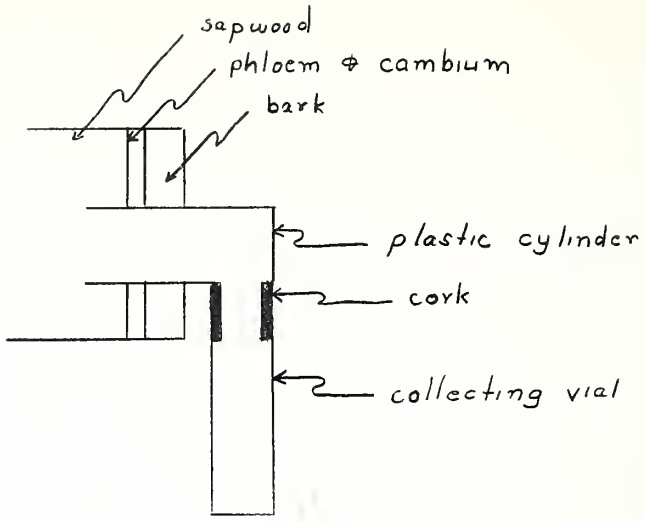

Figure 1.--Diagrammatic cross section of part of tree and resin-collecting device.

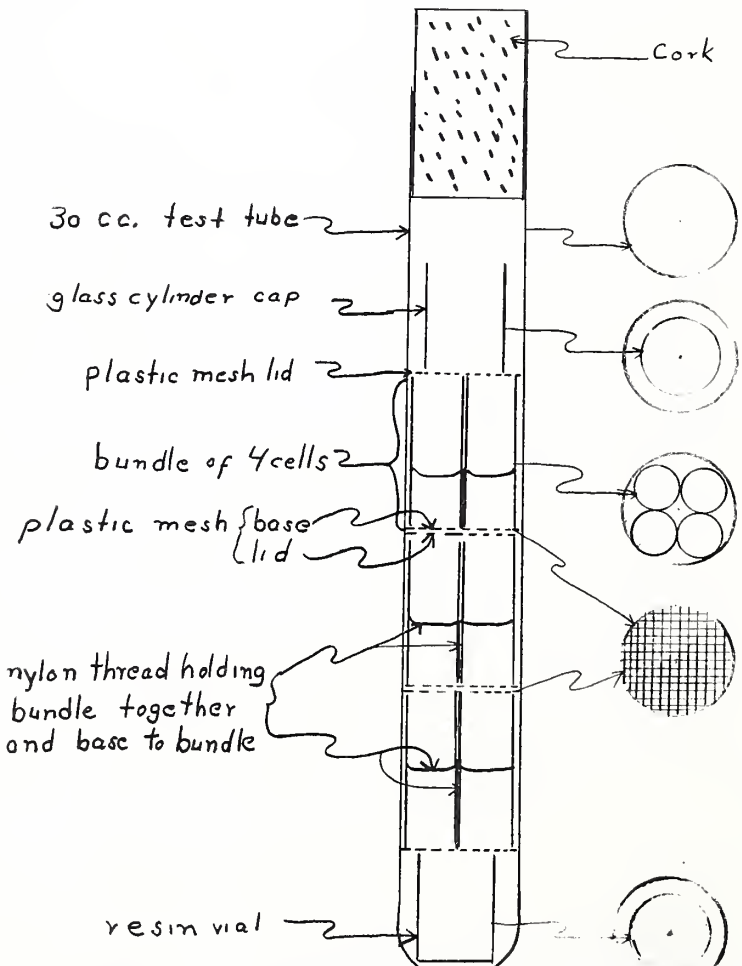

Figure 2.--Longitudinal and cross-sectional diagram of fumigation chamber.

3. The bundles of fumigation cells were placed in the chamber and a disk of 32-mesh lumite plastic screening was placed on top of the bundle. There were 2 or 3 bundles per test tube.

4. After the last bundle was in place, a glass cylinder cap was placed on top of the last disk of plastic mesh.

5. The tube was stoppered with a cork and the chamber was labelled and placed under the conditions of the test.

Recording data

Observations were made at 12- or 24-hour intervals after the test was begun. The primary and usually the only observation was beetle mortality. A beetle was considered dead when it failed to move when it was agitated. Records were maintained individually for each beetle. The sex of each beetle was determined after the test was concluded.

Analysis of data

All results were first plotted, usually with mortality over time. If, by visual inspection, it was considered necessary, an analysis of variance was made by applying the t-test to two conditions of the test. If the visual inspection indicated a clear-cut case of significance or non-significance, no analysis was made. It was only through experience with analysis of similar data that this was possible.

FUMIGANT TOXICITY STUDIES
--TESTS OF RESINS AND RESIN DERIVATIVES--

The four sections under this heading represent the earlier exploratory tests carried out to determine if fumigant toxicity was an area for research.

Ponderosa vs. Jeffrey:

The first test was started on June 26, 1956. At this time techniques and materials had not yet been decided upon; therefore, the test was rather crude. The resin was placed in the bottom of a test tube and a single bundle of 4 beetles placed above it. The test tube was stoppered with cork. Some difficulty was encountered in confining the beetles and some of them were able to get out. In doing so they fell into the resin. The beetles were collected early in the morning and used late in the afternoon. There were three replicates for each condition of the tests, with initially 4 beetles for each replicate. Three dosages were used for ponderosa pine resin and for Jeffrey

pine resin. The test was conducted at a fluctuating room temperature which varied from about 75°F., to 90°F. with the normal change of light and darkness. The results are given in table 1.

Table 1.--Cumulative beetle mortality from confined vapors of ponderosa and Jeffrey pine resin

Resin and dose (ml.)	Total beetles used	Hours after start of test		
		12	24	36
	Number			
Ponderosa pine				
2.0	5	0	3	5
0.2	5	0	1	1
0.02	12	0	2	7
Jeffrey pine				
2.0	12	12	12	12
0.2	11	11	11	11
0.02	10	0	5	7
Untreated	12	0	0	1

A second test, #3, was started on 28th of June and was essentially a repeat of #1. It was made simply to see if the results of #1 could be repeated. One bundle of 4 beetles was used in each test tube and 35 mm. shell vial was used to keep the bundle firmly at one level in the cork-stoppered test tube. The test was carried out at a constant temperature of 72°F, without light. The beetles were collected early in the morning, kept at 40°F. during the day, and used late in the afternoon. Three replicates of 4 beetles each were used for each dosage of each of the two resin, ponderosa and Jeffrey.

The data are found in table 2.

Table 2.--Cumulative beetle mortality from confined vapors of ponderosa and Jeffrey pine resin

Resin and dose (ml.)	Total beetles used	Days after start of test				
		1	2	3	4	5
	Number					
Ponderosa						
1.0	12	0	2	6	10	10
.1	12	0	1	4	8	12
.01	12	0	2	4	9	12
Jeffrey						
1.0	12	12	12	12	12	12
.1	12	2	8	8	9	12
.01	12	0	2	3	6	9
Untreated	12	1	1	1	3	5

A third test, #8, will be only briefly mentioned because of the uncertainty of the conditions. It was basically a second run of the resin of test #3. When #3 was concluded, the beetles were removed and the test tubes recorked immediately and kept at 72°F. for the next 9 days. At that time test #8 was installed by putting fresh beetles into these test tubes. At 84 hours after the test was started, the following mortality counts were made: P.p. at 1.0 = 8; P.p. at .1 = 3; P.p. at .01 = 0; P.j. at 1.0 = 4; P.j. at .1 = 2; P.j. at .01 = 1; untreated check = 0.

Discussion: There are indications that Jeffrey pine resin vapors are highly toxic to the western pine beetle while ponderosa pine is much less so. At this time there was no explanation for the lack of difference between the dosages of ponderosa resin and the wide differences between those of Jeffrey resin.

This procedure should be refined before any reliable conclusions can be reached. The number of beetles per replicate should be increased. The last test indicates that ponderosa resin volatilizes more slowly or has a greater percent volatility, or that the volatile fraction of Jeffrey resin is absorbed by or passes through cork more readily.

Ponderosa vs. Ponderosa

Test No. 5 was started on the 2nd of July to see if there was a difference in the effect of the vapor from the resin of two different

trees of ponderosa pine. All dosages were .1 ml. in a cork-stoppered test tube with 8 beetles per replicate and 6 replicates for each condition of the test. The test was maintained at normal room temperature, 75°-90°F., and light. The results are given in table 3.

Table 3.--Cumulative beetle mortality from confined vapors of resin from different ponderosa pines

Resin source	Replicate number	Total beetles used	Days after start of test				
			1	2	3	4	5
		Number					
Tree #1	1	8	0	4	4	5	8
	2	8	0	5	6	7	8
	3	8	0	4	5	7	8
	4	8	0	2	4	8	8
	5	8	2	5	6	7	8
	6	8	0	1	4	8	8
	Total	48	2	21	29	42	48
Tree #2	1	8	0	2	6	8	8
	2	8	0	3	4	5	8
	3	8	0	3	7	8	8
	4	8	1	1	5	7	8
	5	8	1	6	8	8	8
	6	8	0	5	6	7	8
	Total	48	2	20	36	43	48
Untreated	1	8	0	0	2	3	4
	2	8	0	0	0	3	3
	3	8	0	0	1	4	6
	4	8	0	0	1	3	4
	5	8	0	0	1	3	3
	6	8	0	0	1	5	5
	Total	48	0	0	6	21	25
			t - values				
#1 vs. #2					.2	1.9	
#1 vs. untreated						8.0	

t at 90 percent = 1.8

Discussion: The t-test shows no significant difference between the two sources of resin except at 72 hours. There is an obvious difference between either of the sources of resin and the untreated check. The

large differences between replicates of the same treatment again point to the need for more replicates.

Jeffrey vs. Jeffrey

Test #6 was started on July 4. Its purpose was to find a dosage gradient for Jeffrey pine resin from the same tree. The assembly procedure was that used in the previous tests. The age and condition of the beetles was comparable to that of the other tests. The four dosages of fresh Jeffrey pine resin were .01 ml., .05 ml., .25 ml., and 1.25 ml. There were 8 beetles per replicate and 3 replicates for each dosage and an untreated check. The test was maintained at normal room temperature, 75°F., to 90°F., and light. The results are summarized in table 4.

Table 4.--Cumulative beetle mortality from confined vapors of Jeffrey pine resin

Dose	Replicate number	Total beetles used	Days after start of test					
			½	1	2	3	4	5
Ml.		Number						
.01	1	8	0	0	0	4	7	8
	2	8	0	0	1	2	4	8
	3	8	0	0	0	1	5	8
	Total	24	0	0	1	7	16	24
.05	1	8	0	1	6	8		
	2	8	1	3	7	8		
	3	8	0	3	8	8		
	Total	24	1	7	21	24		
.25	1	8	8	8				
	2	8	7	8				
	3	8	8	8				
	Total	24	23	24				
1.25	1	8	8					
	2	8	8					
	3	8	8					
	Total	24	24					
Untreated	1	8	0	0	0	2	4	6
	2	8	0	0	0	0	0	2
	3	8	0	0	0	0	5	7
	Total	24	0	0	0	2	9	15

Discussion: The t-test shows a significant difference at the 99 percent level between the lowest dosage and the check. Visually there is a significant difference between the various dosages except the two largest. Other data which touch on this factor are found in tables 23 and 27 and illustrated in figures 5 and 9.

Ponderosa vs. supernatant liquid

Test 15 started on the 2nd of August. It was designed to see if there was a difference between the volatile fraction of fresh resin and of the supernatant liquid which results from the partial crystallization of fresh resin. The standard procedure of assembly was used with .4 ml. of the resin or supernatant liquid pipetted into the standard resin vials. There were 12 beetles per replicate and 4 replicates for each condition of the test. The resin and liquid were from ponderosa pine. Two sources of supernatant liquid were used. One resulted from resin standing for 3 days, and the other resulted from resin standing 3 weeks. The test was carried out under normal room light and temperature. The results are given in table 5.

Table 5.--Cumulative beetle mortality from confined vapors of fresh resin and supernatant liquid of ponderosa pine resin

Treatment	Total beetles used	Days after start of test							
		1	2	3	4	5	6	7	8
	Number								
Fresh resin	48	2	11	16	29	39	48		
3-day liquid	48	0	1	5	16	36	41	45	48
3-week liquid	48	0	1	7	21	36	41	44	48
Untreated	48	0	0	0	6	13	27	34	40

Discussion: There is a significant difference between fresh resin and the supernatant liquid during the first 3 or 4 days. One can only speculate on the cause. It could be that some highly volatile material, which is quite toxic, either escapes during the formation of the liquid or is changed or combined with the crystalline fraction during this period. There is no difference between the 3-day- and 3-week-old supernatant liquid.

FUMIGANT TOXICITY STUDIES
----TESTS OF FACTORS----

The twelve sections under this part of the report pertain to various tests, which were conducted throughout the summer, to determine the effect of various controllable factors which might influence the rate of mortality of the beetle or the properties of the resin.

Position of beetle

Test No. 2 was started on 27 June 1956 and was designed to determine the effect of height of the beetles above the resin. The same general procedure was the same as that used in other tests. The dosage was limited to .02 ml. of P.j. resin. The following steps were taken to assemble the test: (1) The resin was pipetted at the bottom of the test tube; (2) a 35 mm. shell vial was then inserted; (3) a bundle of 4 beetles was placed on top of the vial; (4) a second 35 mm. vial was placed on top of the bundle; (5) a second bundle was placed on top of the second vial; and (6) the test tube was stoppered with a cork. In this way 4 beetles in each tube were 35 mm. above the resin while 4 others were 70 mm. above.

There were 5 replicates with resin and 3 replicates to serve as untreated checks. The beetles were collected in the morning, held at 40°F. during the day, and used late in the afternoon. The test was carried out at a fluctuating room temperature of 75-90°F. The results are summarized in table 6.

Table 6.--Cumulative beetle mortality from confined resin vapors at different fumigation chamber positions

Resin and position (mm.)	Total beetles used	Days after start of test: 1	2	3	4	5	6
	Number	---Percent---					
Jeffrey pine							
35	20	12	42	79	83	87	100
70	20	21	46	75	79	83	100
Untreated							
35	12	0	17	25	42	67	75
70	12	0	8	16	50	75	83

F (from 1 to 3 days) between 35 mm. and 70 mm. = 3.29

F at 95 % = 234

Discussion: The F-test shows no difference in mortality between the 35 mm. and the 70 mm. height at several different times. No analysis was made between the resin and untreated check since a visual inspection shows an obvious difference until there is a very high percent natural mortality. There appears to be no difference in the rate of mortality, either in a resinous or untreated atmosphere, between beetles at different heights in the test tube.

Volume of fumigation chamber

Test No. 7, started on 9 July, was designed to determine the effect of different volumes of the fumigation chamber. The standard test tube was used to obtain a 30 cc. volume. A 15 cc. volume was obtained by forcing a cork one-half way down a test tube after the resin and beetles had been placed in it. A long test tube was used for the 60 cc. volume. Two other factors were added to the test. The first was to place a replicate of 4 beetles above each cork in those test tubes in which the volume had been reduced to 15 cc. by forcing a cork halfway. The other was to apportion the resin in one-half of the replicates into separate resin vials. A ratio of 1 volume of resin for 75 volumes of the fumigation chamber was used to obtain an equal resin-to-air dosage for all volumes. Ponderosa pine resin was used for all parts of the test. The beetles were comparable to those used in other tests. The test was maintained at normal room temperature, 75°F. to 90°F., and light. The results are summarized in table 7.

Table 7.--Cumulative beetle mortality from confined resin vapors in fumigant chambers of different volumes

Volume of chamber	Resin apportioned into	Total beetles used	Days after start of test: 1	2	3	4	5
cc.		Number					
15	Test tube	32	8	26	29	31	32
15	Vial	32	4	23	29	31	32
30	Test tube	32	5	21	29	31	32
30	Vial	32	1	19	25	30	32
60	Test tube	32	4	18	26	32	32
60	Vial	32	4	22	31	31	32
Above 15 cc. cork		32	0	7	9	20	30
Untreated		32	0	1	7	7	12

	t - values				
15 vs. 30	1.56	1.24	.68		
15 vs. 60	.83	1.31	.54		
30 vs. 60	.48	1.49	.38		
Untreated vs. above 15 cc. cork			.27	2.132	4.210

t at 90% = 1.943
t at 95% = 2.447

Discussion: The t-test shows no significant difference, even at the 90 percent level, between the three different volumes. Visually there is no difference between the two different ways of apportioning the resin into the test tube. There is a difference between the untreated check and those beetles which were confined to the atmosphere above the cork used to obtain the 15 cc. volume. This could indicate that there was a slow diffusion of vapors through or around the edges of the cork.

Refrigeration of beetles and resin

Test No. 4 was started on the 30th of June and was designed to ascertain the effect of refrigerating beetles and resin prior to use in tests. The last test indicated a need for larger samples, and at the time beetles were not emerging in sufficient numbers to meet this requirement. However, if beetles could be collected over a period of 24 or 48 hours and kept under refrigeration until needed, it would be possible to accumulate enough beetles to make more and larger replicates. Therefore one set of beetles was collected 32 hours before the test was started and was held at 40°F. The other set was collected in the morning and held at 40°F. for about 8 hours until the test was assembled in the afternoon.

At this time difficulty was also being experienced in securing fresh, uncrystallized resin. Therefore, the possibility was considered of getting a large supply of resin and quickly freezing it after apportioning it into desired amounts. In this test the resin for part of the test was obtained 24 hours before the test was started. It was apportioned into test tubes and quickly put in the cold room at 35°F.

The test was carried out and maintained at normal room temperature, 75°-90°F., and light. There were 8 beetles per replicate and 3 replicates for each condition of the test. All dosages were the same, .2 ml. of ponderosa pine resin. With 8 beetles per replicate, the variation between them was reduced. Therefore the results, summarized in table 8, show the totals for the 3 replicates of each condition.

Test #9 was started on the 12th of July to obtain more data on the use of refrigerated beetles. Refrigeration periods of 0, 24, and 48 hours were used with the temperature of refrigeration at about 45°F. (This was not the desired temperature. Shortly before the test was started, the temperature control on the refrigerator began to function improperly. Therefore, though a temperature of 40°F. was desired, the actual temperature realized was about 45°F.) Ponderosa pine resin was used at .4 ml. and 0.4 ml. dosages for the standard 30 cc. volume test tubes. There were untreated checks for each age of refrigeration. The test was maintained at normal room temperature, 75°-90°F., and light. The results are summarized in table 9, and illustrated graphically in figure 3.

Table 8.--Cumulative beetle mortality from confined ponderosa pine resin vapors under different refrigeration conditions

Conditions		Total beetles used	Days after start of test				
Resin	Beetles		1	2	3	4	5
		Number					
Refrigerated	Fresh	24	8	15	19	24	24
Refrigerated	Refrigerated	24	5	10	17	20	24
Fresh	Fresh	24	6	14	20	24	24
Fresh	Refrigerated	24	1	7	16	22	24
Untreated	Fresh	24	0	0	3	9	13
Untreated	Refrigerated	24	0	0	1	5	9

	t-values		
Fresh vs. refrigerated resin	.848	.727	1.120
Fresh vs. refrigerated beetles	1.157	1.781	.217

t at 90% = 1.812

Table 9.--Cumulative mortality of beetles refrigerated prior to exposure to confined ponderosa pine resin vapors

Refrigeration period	Resin dosage	Total beetles used	Days after start of test				
			1	2	3	4	5
Hours	Ml.	Number					
48	.4	24	0	2	15	23	24
24	.4	24	0	2	6	11	18
0	.4	24	0	1	7	17	22
48	.04	24	0	1	13	16	22
24	.04	24	0	1	8	16	22
0	.04	24	0	0	5	11	18
48	0	24	0	1	1	2	6
24	0	24	0	0	1	3	6
0	0	24	0	0	1	1	3

Resinous atmospheres	t - values	
48 hrs. vs. 24 hrs.	0	2.49
48 hrs. vs. 0 hrs.	1.166	2.396
24 hrs. vs. 0 hrs.	1.166	.409

t at 90% = 1.812
95% = 2.228

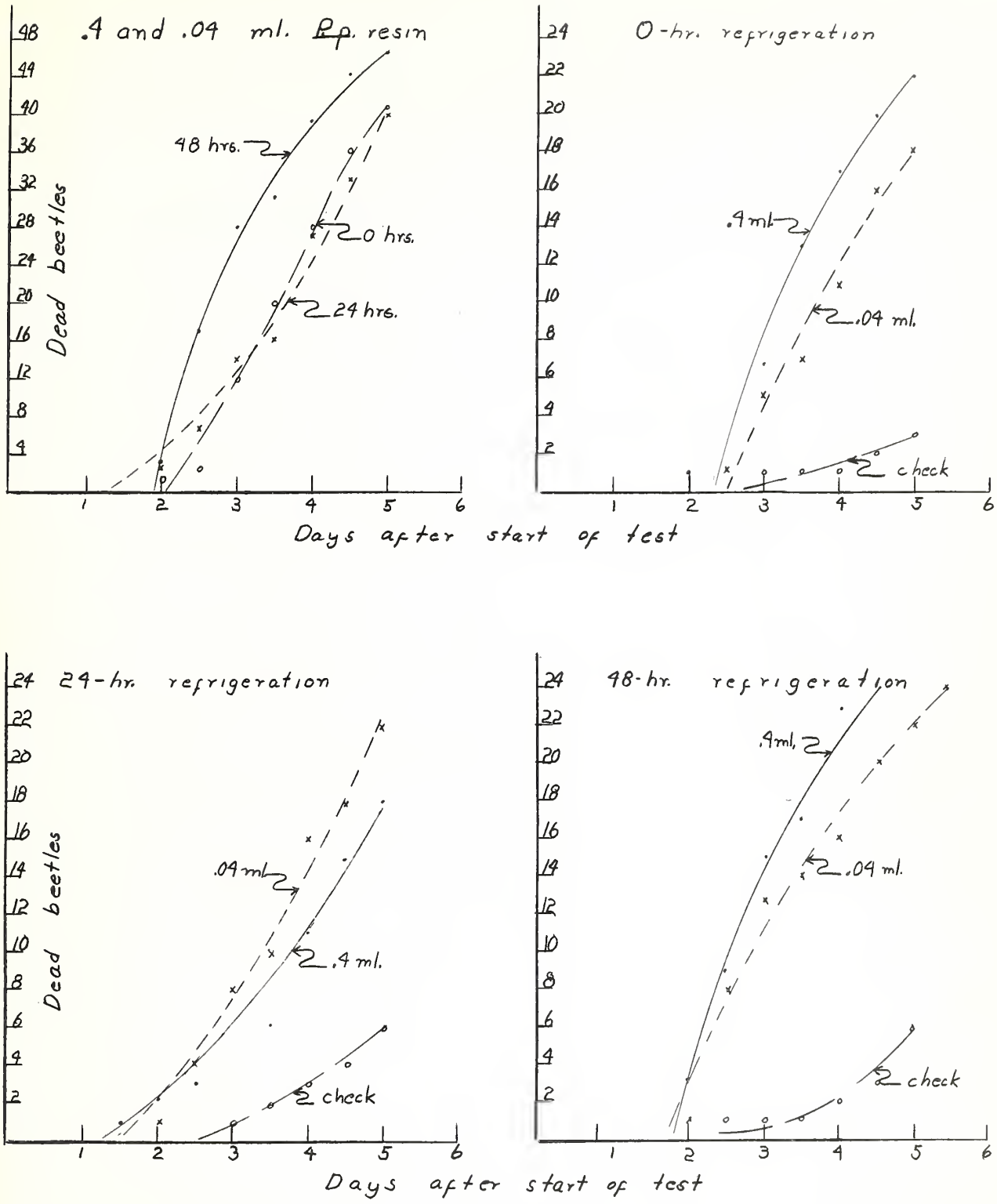

Figure 3.--Effect of the refrigeration of D. brevicomis prior to confinement with P. ponderosa resin vapors.

One part of test No. 14 was to determine the possibility of refrigerating resin for long periods. At the time difficulty was experienced in securing resin when it was needed. At the start, .5 ml. samples of ponderosa pine resin were pipetted into the small resin vials which were immediately corked and placed at 35°F. Then at periodic intervals 5 to 7 samples were removed and used in the standard procedure to test their fumigant effect on the beetle. The lots of resin samples were used at 1, 2, 4, 8, 16 and 32 days after being placed under refrigeration. Unfortunately, the various lots of beetles were not exactly comparable. There were differences in brood source as well as differences in temperature during the period of emergence. Also, since the test was run at normal room light and temperature conditions, there was considerable variation in the conditions under which various parts of the test were conducted. Therefore, only a broad conclusion can be made from the results which are given in summary form in table 10.

Table 10.--Cumulative beetle mortality for confined vapors of ponderosa pine resin refrigerated for different periods prior to use

Resin refrigeration period	Amount of resin	Total beetles used	Days after start of test								
			1	2	3	4	5	6	7	8	9
Days	Ml.	Number	---Percent---								
0	.5	60	3	32	50	72	88	92	97	98	100
	0	60	0	0	2	13	35	60	75	88	98
1	.5	84	0	20	50	70	82	92	100	100	100
	0	84	0	2	2	25	42	67	85	90	92
2	.5	72	0	8	42	70	88	97	98	100	100
	0	72	0	2	6	15	33	53	70	87	97
4	.5	72	19	55	95	100	100	100	100		
	0	72	2	10	19	43	72	90	97		
8	.5	72	27	67	93	100	100				
	0	72	5	19	56	75	90				
16	.5	72	0	27	72	93	98	100			
	0	72	0	0	12	25	50	77			
32	.5	60	0	37	70	85	98	98	100		
	0	60	0	2	13	37	58	75	95		

Discussion: The t-test of the data shows no significant difference, even at the 90 percent level, between 0- and 24-hour-refrigerated beetles or

between the .4 ml. and .04 ml. dosage. By the same test a significant difference at the 95 percent level is indicated between 48-hour and 24-hour-refrigerated beetles. From this it can be concluded that it is safe to use beetles which have been refrigerated for 24 hours while the use of 48-hour-refrigerated beetles entails some risk.

This again shows the lack of a difference in the effect of different dosages of ponderosa pine resin.

In parts of the test there was a tendency for the refrigerated beetles to have a slightly slower rate of mortality than ones which were not refrigerated. This is probably not caused by the refrigeration but by differences in the two lots of beetles.

Because of the great deal of variation from one period of the test to the next, only broad conclusions can be reached regarding the long-time refrigeration of resin. The main one is that resin may be refrigerated to retain the volatile fraction.

Degree of closure of chamber

Test 10 was started on the 12th of July to determine the effect of the degree of closure of the test tube. The three conditions used were (1) a tight cork or 100 percent closure; (2) a cork with a 2/32-inch hole or 97 percent closure; and (3) a cork with a 3/32-inch hole or 94 percent closure. Two dosages, .4 ml. and .04 ml., of P.p. resin, were used along with a set of untreated checks. Three replicates of 8 beetles each were used for each combination of the conditions of the test. The test was maintained at normal room temperature and light. The results are summarized in table 11.

Discussion: The t-test indicates a significant difference at the 90 percent level between 100 percent closure and either of the other two at three days. There is no difference between 94 percent and 97 percent even at the 90 percent confidence level. There appears to be no difference in the untreated check as a result of the degree of closure. However, the test was not maintained for long enough to obtain reliable data.

Again the lack of difference is apparent between the two dosages of ponderosa pine resin.

Time of exposure of beetles to resin vapors

Test 11 was started on the 16th of July to determine the effect of the time of exposure of the beetles to the resinous vapors. Ponderosa pine resin dosages of .3 ml. and 1.0 ml. were used along with untreated checks. A series of replicates was disassembled at 12, 24, 48, and 72 hours, with a series allowed to continue confined with resin for the entire period of the test. In the disassembling, the bundles of

Table 11.--Cumulative beetle mortality from ponderosa pine resin vapors under different degrees of closure of fumigation chamber

Fumigation chamber closure	Amount of resin	Total beetles used	Days after start of test: 2	2½	3	3½	4	4½	5
Percent	ml.	Number							
100, 97, 94	.4	72	4	12	27	37	58	65	72
100, 97, 94	.04	72	3	14	25	31	48	59	68
100	.4 & .04	48	2	12	23	26	36	43	48
97	.4 & .04	48	2	8	16	20	37	40	44
94	.4 & .04	48	3	6	13	22	33	41	46
100	0	24	0	0	0	3	8	12	13
97	0	24	0	0	1	1	3	4	6
94	0	24	0	0	0	1	1	1	3

Resinous atmospheres	t-values			
100% vs. 97%	1.1	1.8	1.5	.2
100% vs. 94%	1.1	2.3	1.2	.7
97% vs. 94%	.3	1.1	.7	1.5

t at 90% = 1.8
95 = 2.2

beetles were removed from the resinous atmosphere in the cork-stoppered test tube and transferred to a clean test tube where the periodic observations were continued. Three replicates of 8 beetles each were used for each combination of conditions of the test. The test was maintained under conditions of normal room light and temperature. The results are presented in table 12.

Discussion: The t-test indicates no significant difference, even at 90 percent confidence level, between 12 and 24 hours, 24 and 48, 24 and 72, and 48 and 72. Significance above 90 percent confidence was obtained between 12 and 48, 12 and 72 and 12 and check. This would suggest that the effect of the vapor is registered between 0 and 24 hours. Additional lengths of exposures had little effect. As with other tests there is no difference between the 2 dosages of resin.

Additional data relative to time of exposure are given in table 19.

Table 12.--Cumulative mortality of beetles exposed for different periods of time to confined ponderosa pine resin vapors

Time of exposure	Amount of resin	Total beetles used	Days after start of test: 1½	2	2½	3	3½	4	5
Hours	ml.	Number							
12	.3	24	3	8	8	14	14	19	21
	1.0	24	2	6	8	17	18	21	23
24	.3	24	0	7	12	16	20	22	22
	1.0	24	5	14	18	18	20	22	24
48	.3	24	2	12	15	19	20	23	24
	1.0	24	3	12	21	24	24	24	24
72	.3	24	7	16	22	22	22	24	24
	1.0	24	2	8	13	18	22	24	24
Continuous	.3	24	7	14	19	21	22	23	23
	1.0	24	7	12	17	20	21	23	24
Continuous	0	24	0	0	5	7	11	16	22

	t-values			
12 hrs. vs. 24 hrs.	1.1	1.7	.5	1.3
12 " vs. 48 "	2.3	3.6	1.8	2.3
12 " vs. 72 "	1.8	2.2	1.8	2.5
12 " vs. check	-	1.3	3.6	2.0
24 " vs. 48 hrs.	.5	.7	1.0	.9
24 " vs. 72 "	.4	.6	.8	1.2
48 " vs. 72 "	.0	.2	.6	.0

t at 90% = 1.8
95% = 2.2

Area and volume of resin

This is the second part of test No. 14 which was started on the 30th of July. Its objective was to determine the effect of different volumes and evaporative areas of resin upon its fumigant effect.

Using the standard procedure, four conditions were established as follows:

1. .5 ml. of resin with a 45 $mm.^2$ evaporating area
2. .5 ml. of resin with a 21 $mm.^2$ evaporating area

3. .25 ml. of resin with a 45 mm.^{2} evaporating area
4. Untreated check

The resin from ponderosa pine was pipetted into small vials with the desired cross sectional area. These were then placed into the 30 cc. test tube. The bundles of beetles were placed above the resin vials and the test tube stoppered with cork. There were 5 replicates of 12 beetles each for each condition of the test. The test was carried out under normal room light and temperature conditions. At the conclusion of the test the sex of each beetle was determined. The results of the sex analysis appears in table 20.

Table 13 summarizes the results.

Table 13.--Cumulative mortality of beetles from doses of ponderosa pine resin having different evaporative areas

Resin		Total beetles used	Days after start of test							
Volume	Area		½	1	2	3	4	5	6	7	8
ml.	mm.^{2}	Number									
.5	45	60	0	2	19	30	43	53	55	58	59
.5	21	60	0	1	15	26	43	53	56	58	59
.25	45	60	0	2	19	28	39	55	59	59	60
Untreated		60	0	0	1	1	8	21	36	45	53

Day	Comparison	t-value
3rd	.5 at 45 mm.^{2} vs. .5 at 21 mm.^{2}	= 1.600
	.5 at 45 mm.^{2} vs. .25 at 45 mm.^{2}	= .571
	.5 at 21 mm.^{2} vs. .25 at 45 mm.^{2}	= .500

t at 90% = 1.860

Discussion: Again, differences in volume and area of ponderosa pine resin had no effect on the fumigant properties of the resin.

Additional data relevant to volume of resin are presented in tables 19, 23, 25, 26, 27 and figure 9.

Temperature

Test 16 was the first one designed to assay the role of temperature. It was started on the 1st of August. The dosage was .4 ml. of ponderosa pine resin pipetted into resin vials. The tubes were assembled immediately. There were 5 replicates of 12 beetles each for each of the two temperatures, 36°C. and 20°C., with an equal number of untreated checks for each temperature. The results are tabulated in table 14.

Table 14.--Cumulative beetle mortality from confined vapors of ponderosa pine resin under different temperature conditions

Amount of resin	Temp.	Total beetles used	Days after start of test									
			1	2	3	4	5	6	7	8	9	10
ml.	°C.	Number										
.4	20	60	0	9	28	29	37	45	48	53	55	57
0	20	60	0	0	1	2	8	24	34	40	43	49
.4	36	60	32	56	57	60						
0	36	60	0	5	16	31	42	56	60			

Test 17, started on the 14th of August, was an enlargement of No. 16 in that three different temperatures were used: 21.6 C., 25.8°C., and 27.3°C. The aim was to have a 4° interval but the temperature control units were not that exact. Therefore, the temperatures are the average for the period of the test. Again, .4 ml. ponderosa pine resin was used in the resin vials. There were 5 replicates of 12 beetles each. The results are given in table 15.

Table 15.--Cumulative beetle mortality from confined vapors of ponderosa pine resin under different temperature conditions

Temp.	Amount of resin	Total beetles used	Days after start of test											
			1	2	3	4	5	6	7	8	9	10	11	12
°C.	ml.	Number												
21.6	.4	60	1	5	11	27	36	41	47	51	57	58	60	
25.8	.4	60	1	10	32	47	53	59	60					
27.4	.4	60	0	6	14	43	55	60						
21.6	0	60	1	2	2	4	7	14	28	33	35	37	43	48
25.8	0	60	0	0	2	4	13	24	31	38	49	55	59	59
27.3	0	60	0	2	2	5	9	20	33	38	46	50	56	59

	t-values				
Resinous atmospheres					
21.6°C. vs. 25.8°C.	1.6	8.9	3.6	4.1	7.1
21.6 vs. 27.3	.4	1.0	5.4	5.2	10.2
25.8 vs. 27.3	1.0	5.1	1.3	.7	1.3

t at 90% = 1.9
95% = 2.3

Discussion: It is obvious that that temperature has a marked effect on the mortality rate of the beetle. Variations in temperature during the test may invalidate comparisons between 25.8°C. and 27.3°C.

Though an accurate determination cannot be made, it would appear that the difference between treated beetles at two temperatures is somewhat proportional to the differences in the untreated check. Therefore, the main effect of temperatures may be on natural mortality rather than on the action of the resin. More refined tests will have to be made before this statement can be made without so much uncertainty.

Additional data touching on temperature are given in tables 23, 24, and 27 as well as in figures 6 and 7.

Light

Test 18, started on the 16th of August, was a small one which involved only the factor of light.

The test procedure was standard using .5 ml. of ponderosa pine resin. Half of the test was conducted under normal cyclic conditions of daylight and dark while the remaining half was conducted under conditions of total darkness. The temperature was the same for both halves, being the normal room temperature. There were 4 replicates of 12 beetles for each of the light conditions, with an equal number of replicates for untreated checks. A summary of the results is given in table 16.

Table 16.--Cumulative beetle mortality from confined vapors of ponderosa pine resin under different light conditions

Light conditions	Amount of resin	Total beetles used	Days after start of test									
			1	2	3	4	5	6	7	8	9	10
	ml.	Number										
Diurnal	.5	48	5	18	31	43	47	48				
Dark	.5	48	1	13	24	38	45	45				
Diurnal	0	48	0	0	1	7	13	21	29	33	35	35
Dark	0	48	0	0	4	11	15	20	26	38	41	46
			t-values									
Resinous atmosphere												
Dark vs. diurnal			.69	.67	.67							

t at 90% = 1.943

Discussion: In both the treated and untreated parts of the test, there is no apparent effect of light.

Closure material for chamber

Test 19 was a small one also. It was started on the 17th of August and was a test of closure material for the test tube. Rubber stoppers were used for comparison with the standard cork stoppers. The dose was .5 ml. of ponderosa pine resin and the test was conducted under conditions of normal room light and temperature. Each condition of the test had 4 replicates of 12 beetles each. There were no untreated checks. The results are given in table 17.

Table 17.--Cumulative beetle mortality from ponderosa pine resin vapors confined with different closure materials

Closure material	Amount of resin	Total beetles used	Days after start of test				
			1	2	3	4	5
	ml.	Number					
Rubber stopper	.5	48	24	48			
Cork stopper	.5	48	1	22	39	47	48

Discussion: It is difficult with such limited data to draw reliable conclusions. The great difference between the two conditions of the test might indicate that (1) the cork was not fully stoppering the tube and therefore some of the volatile materials were escaping; (2) by stoppering so completely, the rubber allowed no gas exchange and therefore the mortality was caused by the lack of oxygen; or (3) there was a reaction between the rubber and the volatile fraction of resin which resulted in a highly toxic by-product.

Weight of vapor in chamber

Test 20 was started on August 20. It was becoming quite obvious at this time that volumetric measurement of resin was not sufficiently accurate. Likewise such measurements were not indicative of the effective amount of resin, i.e., the volatile fraction. Therefore the purpose of this test was to determine the volatile amount of resin which accumulated in the standard cork-stoppered test tube and to associate mortality with the volatile fraction and not the gross resin. The amount which volatilized over a certain period of time was also considered.

The procedure was briefly as follows. The standard set-up was used except that the original weight of ponderosa pine resin was obtained. The resin and the beetles were assembled in the 30 cc. test tube. There were 8 lots of resin at approximately .5 ml. volume and an equal number at .1 ml. Two of each of these were reweighed at 15, 24, 48, and 72 hours to find the loss of weight and therefore the amount which had volatilized during the given period of time. Since it was necessary to disassemble the test tube to reweigh the resin, the bundles of beetles were shifted to clean test tubes. In this way the additional factor of time of exposure to vapors was part of the test. The test was carried out at normal room temperature and light using 12 beetles per replicate.

Table 18 is the data obtained from the weighings while table 19 is the mortality recordings.

Table 18.--P. ponderosa resin weight and weight loss in fumigation chamber

15-hr. samples		24-hr. samples		48-hr. samples		72-hr. samples	
Gross wt.	Loss	Gross wt.	Loss	Gross wt.	Loss	Gross wt.	Loss
			Grams				
.4604	.0010	.4626	.0030	.5064	.0031	.6244	.0050
.5476	.0026	.5096	.0032	.4950	.0026	.3332	.0074
.1122	.0014	.0876	.0024	.0862	.0034	.0854	.0044
.0828	.0012	.0894	.0028	.0870	.0042	.0848	.0052

Discussion: The first thing which became obvious is that the amount of resin has little to do with the amount of vapor. Therefore the weights resulting from the .5 ml. and .1 ml. volume apportionments have been grouped by time. There appears to be a rapid loss of resin weight during the first 24-hour period. After this, the rate of loss decreased very rapidly. The phenomenum observed under 1 explains the repeated observations in early tests that the amount of resin in the test tube had little to do with the resulting mortality rate.

As in the earlier test, there appears to be little increase in the toxic effect resulting from increased time of exposure after 24 hours. The most significant effect of the resin on the beetle appears to be registered within the first 24 hours.

Additional data on weight of resin vapors are given in tables 25 and 27.

Table 19.--Cumulative beetle mortality from different amounts of confined ponderosa pine resin vapors and different periods of exposure

Exposure time	Amount of vapor	Total beetles used	Days after start of test: 1	2	3	4	5	6	7	8	9
Hours	Grams	Number									
15	.0010	12	0	2	4	4	7	8	8	11	12
	.0026	12	0	1	4	5	7	9	10	11	12
	.0014	12	0	2	2	5	5	10	10	10	12
	.0012	12	1	3	3	5	9	10	10	11	12
Av.	.0015 Total	48	1	7	13	19	28	37	38	43	48
24	.0030	12	2	4	6	10	11	11	11	12	12
	.0032	12	0	4	6	8	10	11	11	11	12
	.0024	12	0	3	7	9	11	12	12	12	12
	.0028	12	0	2	10	12	12	12	12	12	12
Av.	.0028 Total	48	2	13	29	39	44	46	46	47	48
48	.0031	12	0	4	7	8	11	11	12		
	.0026	12	1	4	11	12	12	12	12		
	.0034	12	0	2	7	10	12	12	12		
	.0042	12	1	1	3	12	12	12	12		
Av.	.0033 Total	48	2	11	28	42	47	47	48		
72	.0050	12	2	7	9	12	12				
	.0074	12	1	4	8	11	12				
	.0044	12	0	2	10	12	12				
	.0052	12	2	4	8	11	12				
Av.	.0055 Total	48	5	17	35	46	48				
Untreated	-	12	1	2	3	3	9	11	12	12	
	-	12	0	0	0	3	10	11	11	12	
	-	12	0	0	1	2	7	10	11	12	
	-	12	0	0	3	5	8	11	11	12	
	- Total	48	1	2	7	13	34	43	45	48	

	t - values			
15 hrs. vs. 24 hrs.	2.8	3.8	5.6	4.4
15 hrs. vs. 48 hrs.	1.3	2.2	5.8	5.6
15 hrs. vs. 72 hrs.	2.4	8.1	18.8	6.1
15 hrs. vs. untreated	2.2	1.7	2.2	1.4
24 hrs. vs. 48 hrs.		.1	.6	1.6
24 hrs. vs. 72 hrs.		1.4	1.7	2.4
48 hrs. vs. 72 hrs.		1.0	.8	

t at 90% = 1.7
95% = 2.0

Use of auxiliary solvents and feeding

Tests 12 and 13 will be reported together. They were started on the 18th and 19th of July.

Test 12 was an attempt to allow beetles to feed while in the presence of resinous vapors. The technique was not suitable and the test was discarded.

Test 13 was an attempt to use auxiliary solvents to keep resin from crystallizing. Both alcohol and acetone were used, and the fumes of both were found to be extremely toxic to the beetles. Therefore, the use of auxiliary solvents was discarded. However, with continued effort, it was possible at this time of the season to secure fresh, uncrystallized resin by other means.

Sex

Parts of three tests were devoted to the analysis of the factor of sex with respect to fumigant mortality. The data derived from them are given in tables 20, 21 and 22. Figure 3 is a graphic presentation of the data in table 21.

Table 20.--Cumulative mortality of male and female beetles from confined vapors of ponderosa pine resin 1/

Treatment and sex	Total beetles used	Days after start of test: ½	1	2	3	4	5	6	7	8
	Number	---Percent---								
Treated beetles										
Females	91	0	1	25	42	57	86	93	98	100
Males	89	0	6	34	52	73	95	98	100	100
Untreated beetles										
Females	27	0	0	4	4	15	33	52	67	85
Males	33	0	0	0	0	12	36	67	82	91
Treated females vs. males		t-values	1.5	1.3	2.0	1.4				
		t at 95%			2.0					

1/ Derived from data in table 13.

Table 21.--Cumulative mortality of male and female beetles from confined vapors of ponderosa pine resin 1/

Treatment and sex	Total beetles used	Days after start of test									
		1	2	3	4	5	6	7	8	9	10
	Number										
Treated beetles											
Females	244	12	75	148	189	225	234	242	244		
Males	249	23	93	177	213	236	244	248	249		
Untreated beetles											
Females	247	0	10	25	50	100	156	199	226	236	247
Males	245	0	14	55	97	153	202	229	239	245	245

1/ Derived from data in table 10.

Table 22.--Cumulative mortality of male and female beetles from confined vapors of ponderosa pine resin 1/

Sex	Total beetles used	Days after start of test									
		1	2	3	4	5	6	7	8	9	10
	Number										
Female	93	5	15	40	63	74	82	84	89	92	93
Male	97	5	33	66	83	93	96	96	97	97	97

1/ Derived from data on treated beetles in table 19.

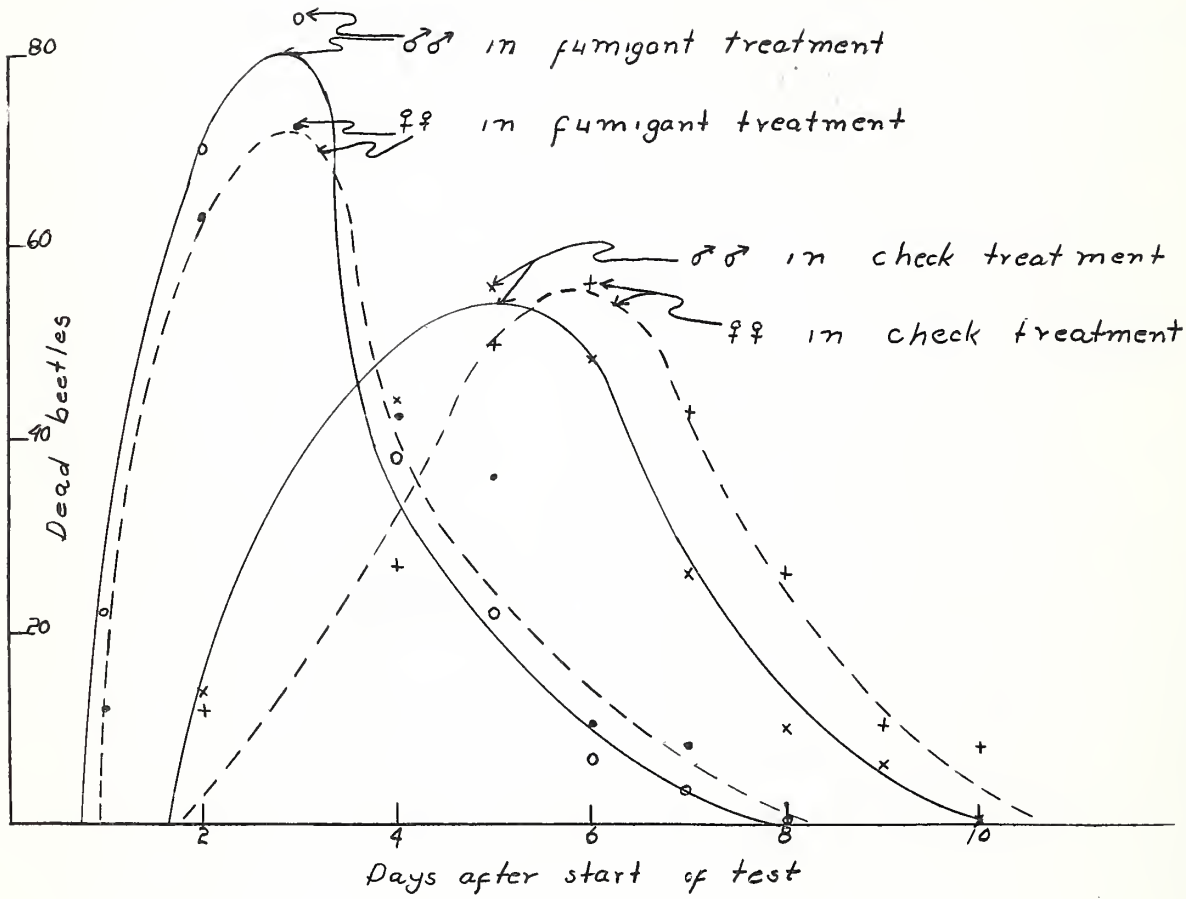

Figure 4.--Effect of sex on mortality rate of D. brevicomis.

Discussion: In all cases the mortality rate of the male is faster than that of the female. The difference between the sexes in a resinous atmosphere is distinctly parallel to that in a non-resinous atmosphere. Likewise the magnitude of the difference between sexes in a resinous atmosphere is quite similar to that in a non-resinous atmosphere.

From this data it would appear that sex can be ignored as a significant factor in resin toxicity tests. Though not recorded in the data, the factor of size may be linked with sex, the females being slightly larger than the males. Thus the differential rate of mortality which can be attributed to sex may actually be a function of size.

Additional data on the factor of sex are given in figures 8, 10, 11.

FUMIGANT TOXICITY STUDIES
--TESTS OF RESINS AND FACTORS--

The three sections which follow represent the final studies of the season. Their purpose was to compare the effect of various pine resins on D. brevicomis, under the best conditions indicated by previous work.

Ponderosa vs. Jeffrey vs. temperature

Test 21 was, in contrast to most of the previous ones, a rather extensive test. Its purpose was to compare the two resins at three temperatures. It was necessary to start different parts of it on different days: the 31st of August and the 2nd and 4th of September. These three days were used to divide the test into 3 equal parts, A, B, C respectively, according to the three temperature conditions, 68°F., 78°F., 88°F. At each temperature, both ponderosa and Jeffrey resin were used at 3 volumetric dosages, 1.0, .2, and .04 ml., in the standard cork-stoppered test tube. There were 6 replicates of 12 beetles each for each condition of the test. Since a different lot of beetles was used for the 3 temperatures it is not possible to compare, without qualification, the results between temperatures. On the 10th day after the test was assembled it was disassembled and the resin weighed. With the previous weights which had been made, it was possible to determine the weight of vapors which had volatilized from the resin over a ten-day period.

As the 78°F. and 88°F. sections of the test were established, a complete set of 6 replicates of untreated check beetles, which were used at each of these temperatures, was placed at 68°F. This made it possible to compare the natural mortality of the beetles used on the three different days.

The composite summary of mortality is given in table 23 while table 24 is a summary of the untreated check mortality at 68°F. for the three sections of the test. Table 25 is a summary of the loss-of-weight determinations.

Figures 5,6,7, and 8 present graphic interpretations of various aspects of the data.

Table 23.--Cumulative beetle mortality from confined vapors of ponderosa and Jeffrey pine resin under different temperature conditions

Temperature and dose	Total beetles used	Days after start of test: 1	2	3	4	5	6	7	8	9	10	11
	ml. Number											
						Ponderosa pine						
68°F. 1.0	72	0	0	2	6	16	34	44	55	62	69	72
0.2	72	0	0	0	3	8	30	47	55	65	70	71
0.04	72	0	0	0	4	13	21	37	50	57	66	67
0	72	0	0	0	1	2	3	12	19	28	39	52
78°F. 1.0	72	0	2	24	42	55	65	68	71	72	72	72
0.2	72	0	0	14	37	55	64	70	70	71	72	72
0.04	72	0	0	15	31	44	60	67	71	71	72	72
0	72	0	0	4	6	17	33	43	51	63	67	72
88°F. 1.0	72	4	34	61	71	72	72					
0.2	72	4	39	68	70	72	72					
0.04	72	2	50	65	72	72	72					
0	72	0	5	31	53	67	72					
						Jeffrey pine 1/						
68°F. 1.0	72	64	72									
0.2	72	30	72									
0.04	72	0	1	1	2	7	9	16	28	43	51	58
78°F. 1.0	72	72										
0.2	72	72										
0.04	72	0	8	18	31	42	63	70	71	72		
88°F. 1.0	72	72										
0.2	72	72										
0.04	72	14	33	48	68	72						

.04 ml. dosage	t-values				
68°F.					
Ponderosa vs. check	1.8	5.0	4.9	4.6	4.5
Jeffrey vs. check	1.3	2.3	2.1	1.6	3.0
Ponderosa vs. Jeffrey	.3	2.9	2.8	3.6	
78°F.					
Ponderosa vs. check	3.4	3.4	3.6	5.9	6.0
Jeffrey vs. check	1.3	2.9	3.0	5.4	7.1
Ponderosa vs. Jeffrey	.3	.0	.4	.6	6.4
88°F.					
Ponderosa vs. check 1.3	7.5	3.4	2.7		
Jeffrey vs. check	2.4	1.3	2.1		
Ponderosa vs. Jeffrey	1.3	1.4	3.0		

t at 90% = 1.8
95% = 2.2

1/ Untreated checks are same as for ponderosa.

Table 24.--Cumulative mortality at 68°F. of untreated beetles collected on 3 different days

Collection	Total beetles used	Days after start of test: 3	4	5	6	7	8	9	10	11	12	13
	Number											
A	72	0	1	2	3	12	19	28	39	52	56	63
B 1/	72	1	1	4	10	16	27	41	58	63	63	67
C 2/	72	0	0	2	6	13	25	38	47	64	65	69

1/ and 2/ Some population of beetles used for 78°F. and 88°F. sections, respectively, in table 23.

Table 25.--Mean weight loss of ponderosa and Jeffrey pine resin in 10 days at different temperatures 1/

Temperature	Ponderosa: 1.0 ml.	.2 ml.	.04 ml.	Jeffrey: 1.0 ml.	.2 ml.	.04 ml.
°F.			Grams			
68	.0073	.0074	.0051	.0265	.0151	.0016
78	.0066	.0074	.0043	.0323	.0181	.0024
88	.0067	.0065	.0047	.0285	.0174	.0027

1/ Resin samples are those used in table 23, each mean weight loss based on 6 samples.

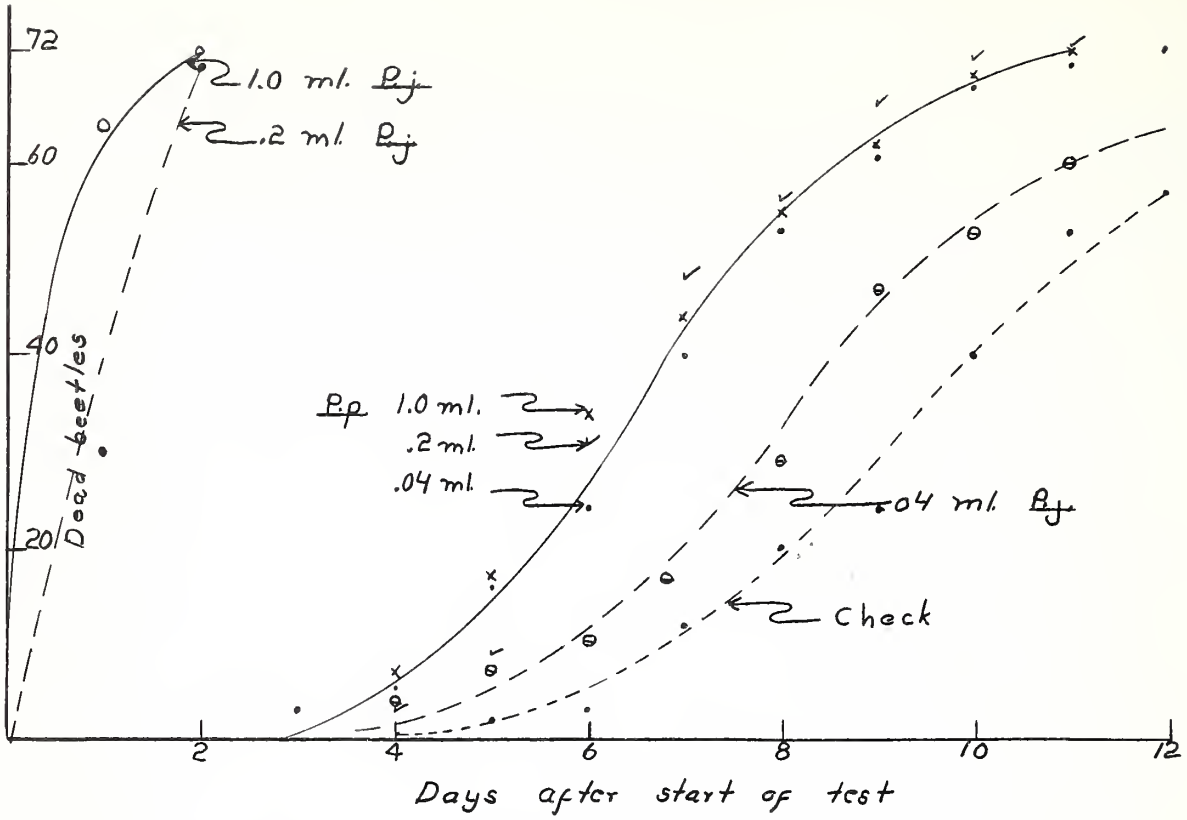

Figure 5.--Effect on D. brevicomis of confined resins of P. ponderosa and P. jeffreyi at 68°F.

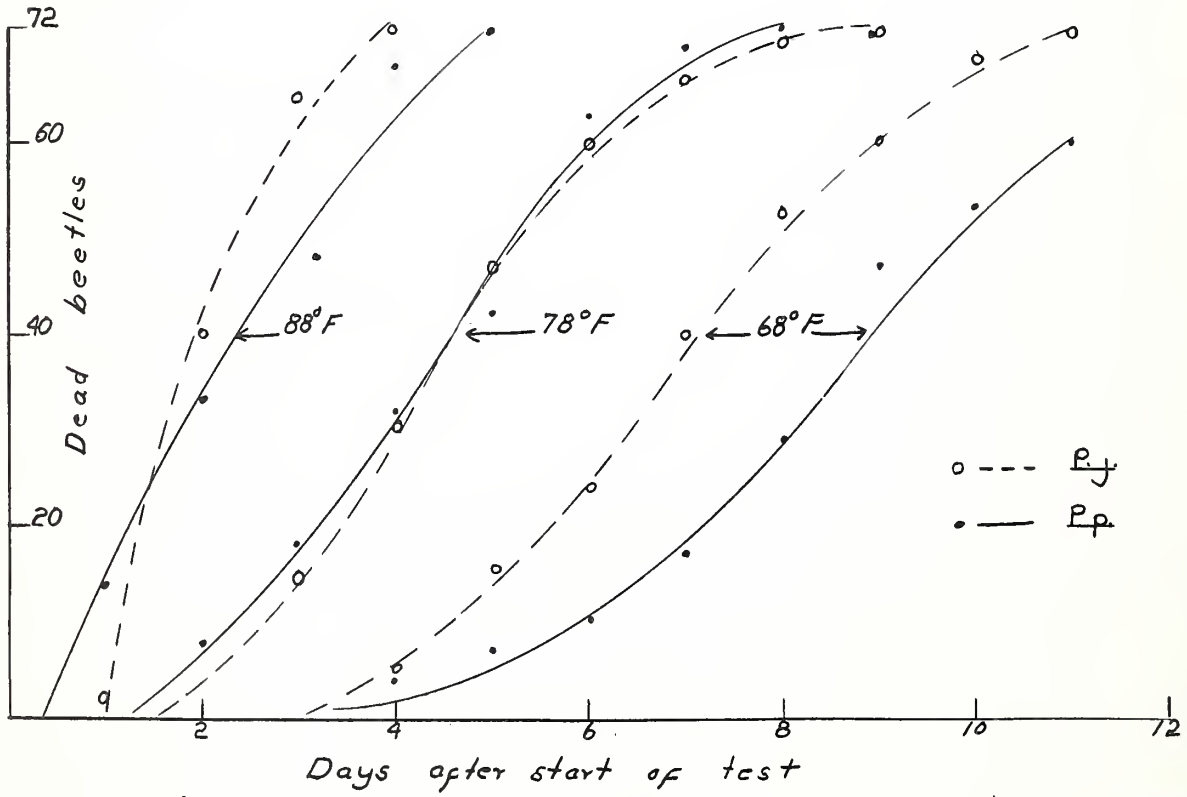

Figure 6.--Effect on mortality rate of D. brevicomis of .04 ml. confined resin of P. ponderosa and P. jeffreyi.

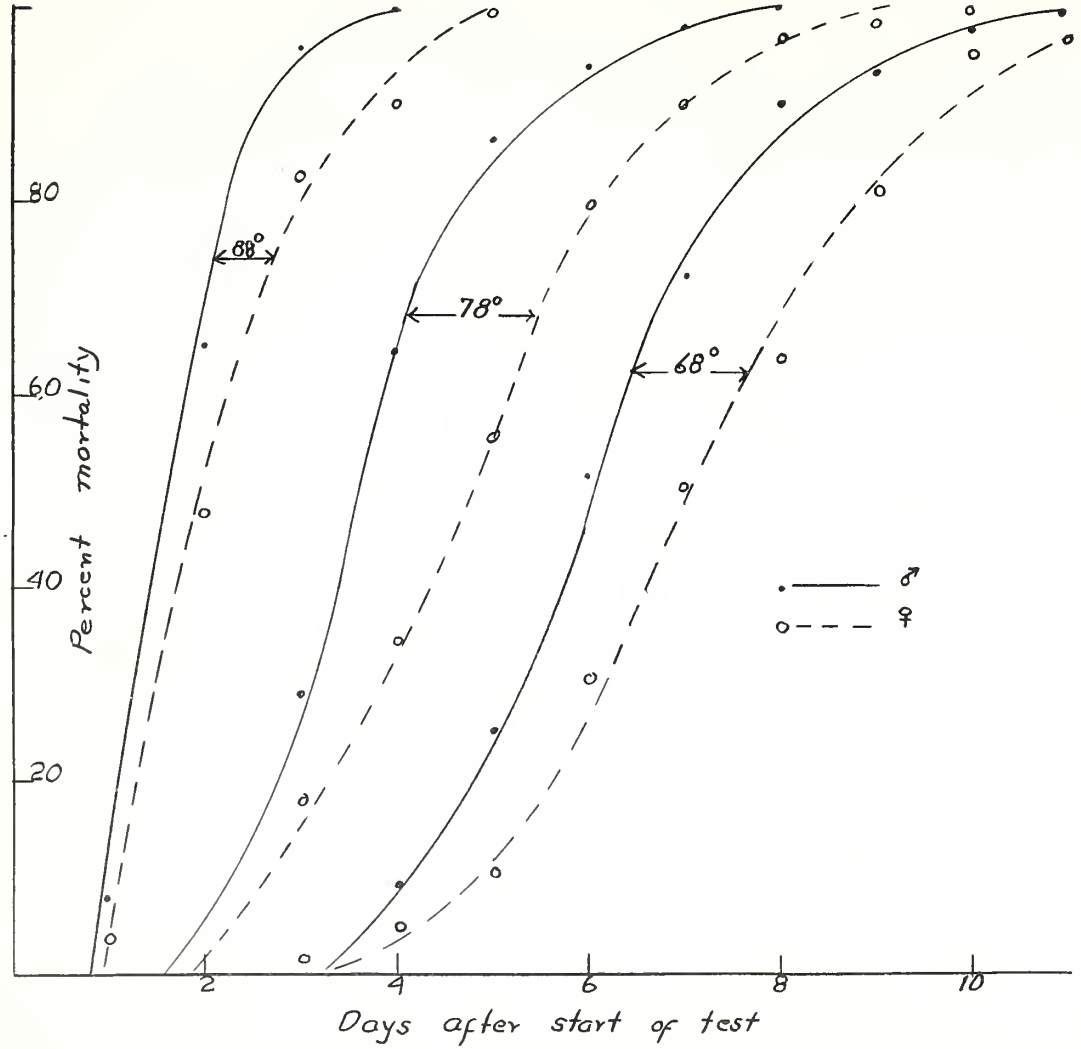

Figure 7.--Effect of sex on mortality rate of D. brevicomis using confined resin of P. ponderosa at different temperatures °F.

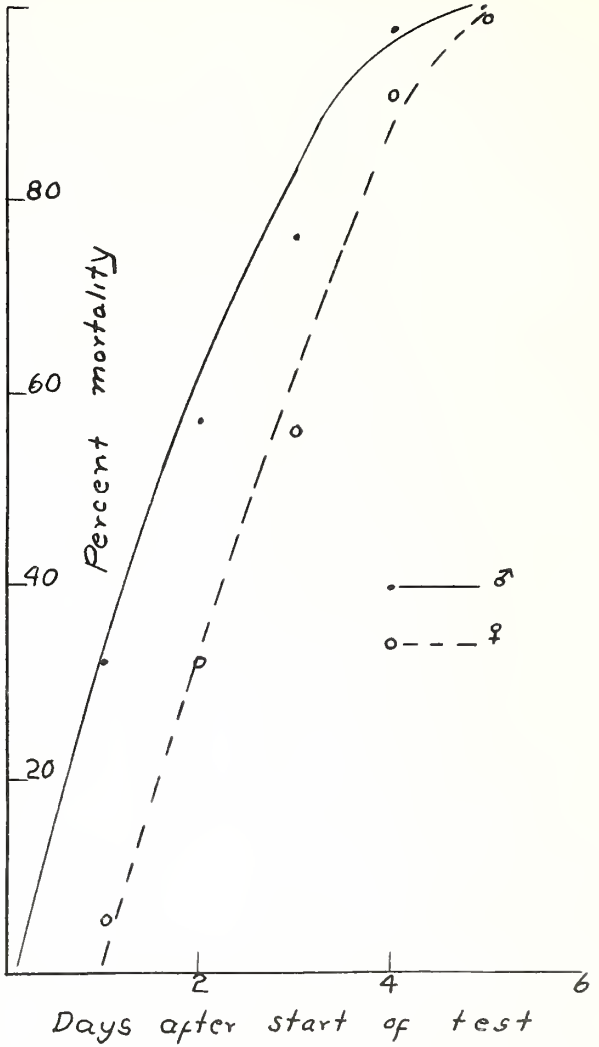

Figure 8.--Effect of sex on mortality rate of D. brevicomis in confined vapors of P. jeffreyi.

Discussion: The major effect of temperature on rate of mortality is again quite evident. It is possible that temperature has a direct arithmetic effect on the mortality rate in the presence of a resinous atmosphere and likewise in a resin-free atmosphere. The effect due to sex is parallel to the general trends reported elsewhere in this report, with the female mortality rate always slightly less than that of the male.

The lack of appreciable difference due to variable dosages of ponderosa resin is again evident. However, the weight-loss aspect of the test shows that this is caused by near-equal loss of resin irregardless of dosage and temperature. On the other hand, the wide difference in effect caused by difference in dosage of Jeffrey resin is explained by the difference in loss of resin attributable to different dosages. The Jeffrey resin weight-loss was not appreciably affected by temperature.

There was a slight difference in the rate of natural mortality between the three lots of beetles. However, the differences are of such a small magnitude that relative comparisons between the 3 parts of the test can be made.

The rather wide variations in weight loss within the various conditions in the test suggest that the cork may be absorbing the resin vapors at a differential rate.

At the lowest dosage, .04 ml., ponderosa and Jeffrey resin are most comparable in weight loss. At this dosage there is frequently a significance between their effect on the beetle.

Resin weight loss vs. temperature

On September 13 and 15 tests were started to determine the loss of weight of ponderosa and Jeffrey resin at different volumetric dosages and at different temperatures. The temperature and dosage conditions were the same as those used in the first part of the section (table 23). The procedure was to apportion the resin volumetrically to approximate the weight of resin desired. Weighings were made to obtain the exact weight of each sample. These were immediately placed in test tubes and stoppered with cork. The test tubes were placed at the desired temperature, and at the prescribed interval a series of tubes were removed and disassembled. By reweighing the resin the amount which had volatilized during the given period of time was measured. The series was then discarded. The results are summarized in table 26.

Discussion: These results show that varying volumetric amounts of ponderosa pine resin produced the same vapor concentration in the fumigation chamber. This explains to a large degree the results of many of the early tests in which quite similar mortality rates were obtained with different resin volumes. With Jeffrey pine resin the difference in mortality rate caused by different volumes of resin are explained. The vapor concentration resulting from volumes of .2 ml. and .04 ml. were approximately

Table 26.--Mean weight loss of confined ponderosa and Jeffrey pine resin for different times and temperature 1/

Ponderosa

Temperature	1.0 ml.		.2 ml.					.04 ml.	
	2-day	4-day	1-day	2-day	2-day 2/	4-day	8-day	2-day	4-day
°F.	Grams								
68				.0024		.0041			
78	.0025	.0042	.0016	.0022	.0026	.0046	.0048	.0022	.0039
88				.0032		.0051			

Jeffrey

Temperature	1.0 ml.		.2 ml.					.04 ml.	
	2-day	4-day	½-day	1-day	2-day	4-day	8-day	2-day	4-day
°F.	Grams								
68					.0119	.0104			
78	.0134	.0158	.0098	.0094	.0132	.0150	.0239	.0056	.0046
88					.0140	.0168			

1/ Each average weight loss based on 5 samples.

2/ Evaporating area ½ standard.

.0130 gram and .0050 gram respectively. Likewise the lack of difference between volumes of .2 ml. and 1.0 ml. is explained by the rather similar vapor concentration which was produced by these dosages.

Though there was an increase in vaporization due to an increase in temperature, it does seem large enough to account for the great difference in mortality due to temperature. Therefore increase in temperature would seem to have a greater effect on the natural mortality rate than on the action of the resinous vapors. It can be seen that in general the magnitude of the difference between natural and treatment mortality was quite comparable at different temperatures.

The slight increase in the loss of weight of resin with an increase in time could be an indication that the concentration of the vapors increased with time. However, in retrospect, it is more than likely indicative of the absorptive action of the cork.

The results of this test were quite instrumental in determining the course of work the following season, since they indicate that the vapor concentration is the factor to be determined rather than a volumetric measurement of the resin. Likewise, the result indicates that careful attention should be given to proper confinement of the resin vapors.

Because of the uncertain nature of the reaction between resin vapors and cork, much of the work on resin weight loss must be accepted with considerable reservation.

Ponderosa vs. Jeffrey vs. Jeffrey x ponderosa

Test No. 23, started on the 23rd and 24th of September, was the concluding experiment in the series of fumigant toxicity tests. Its purpose was to compare the effect on D.b. of confined vapors of the resin of ponderosa pine, Jeffrey pine, and the Jeffrey x ponderosa hybrid. The standard procedure was used with two volumetric dosages of each resin, 1.0 ml. and .1 ml. There were 6 replicates of 12 beetles each for each condition of the test. The test was carried out at 68°F. The summary of results is given in table 27 and graphically presented in figure 9. This was the first opportunity to make an analysis of influence of sex in the response of the beetle to the hybrid resin. These data are given graphically in figure 10.

Table 27.--Cumulative beetle mortality from confined vapors of resin of different pine species

Kind and amount of resin	Total beetles used	Days after start of test												
		½	1	2	3	4	5	6	7	8	9	10	11	12
	ml. Number													
Ponderosa	1.0 72	0	0	7	24	42	54	66	72					
Jeffrey x ponderosa	1.0 72	0	2	54	65	72								
Jeffrey	1.0 72	62	72											
Untreated	0 72	0	0	0	1	4	19	31	41	57	68	70	71	72
Ponderosa	.1 72	0	0	6	19	31	43	61	70	71	71	72		
Jeffrey x ponderosa	.1 72	0	2	27	51	62	67	69	72					
Jeffrey	.1 72	-	72											
Untreated	0 72	0	0	0	3	5	15	32	60	68	72			

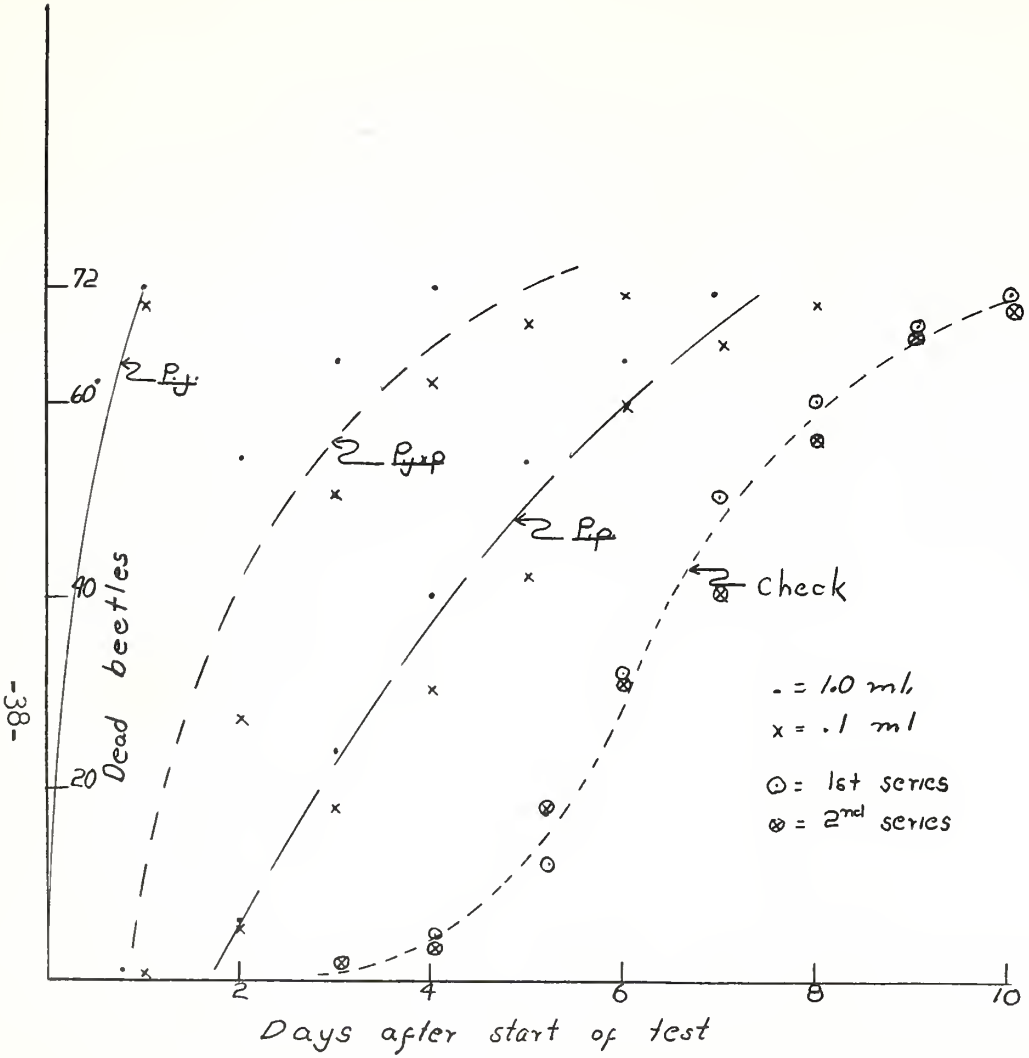

Figure 9.--Effect on D. brevicomis of confined resins of P. ponderosa, P. jeffreyi, and P. jeffreyi x ponderosa.

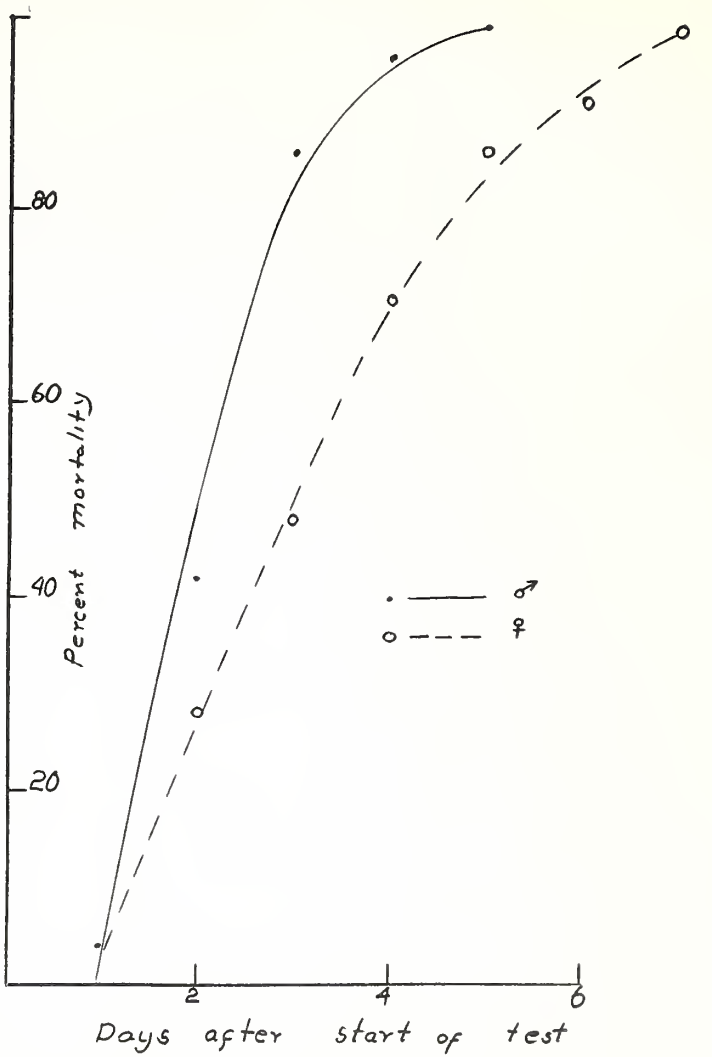

Figure 10.--Effect of sex on mortality rate of D. brevicomis using confined resin of P. jeffreyi x ponderosa.

Discussion: This was the first test in which resin from the Jeffrey x ponderosa hybrid was used, and the results indicate that its effect is intermediate between the two parent trees. Likewise, as in the case of the resin of both parent trees, the females had a less rapid rate of mortality than the males.

SEMI-FUMIGANT TOXICITY STUDIES

Test no. 22 was started on the 21st and 22nd of September. It was designed to determine the effect of semi-confined vapors of ponderosa and Jeffrey resin. The standard procedure was used except the test tubes were not stoppered. The resin vial was placed in the bottom of the test tube and the bundles of beetles were placed above it with the end of the test tube open. Two dosages 1.0 ml. and .1 ml. were used for each of the two pine resins, ponderosa and Jeffrey. Each condition of the test was composed of 6 replicates of 12 beetles each with a set of replicates to serve as an untreated check. The test was conducted at normal room light and temperature. The results are given in table 28.

To see if there was a difference in response due to sex under these conditions, the mortality rate by sex is given in figure 11. The data for the two dosages for each resin have been added together since there is little difference between them.

Table 28.--Cumulative beetle mortality from semi-confined vapors of ponderosa and Jeffrey pine resin

Resin		Total beetles used	Days after start of test									
Kind	Amount		1	2	3	4	5	6	7	8	9	10
	ml.	Number										
Ponderosa	1.0	72	0	5	16	47	59	68	72			
Jeffrey	1.0	72	2	5	9	27	37	48	61	67	72	
Ponderosa	.1	72	0	3	16	32	43	62	67	71	72	
Jeffrey	.1	72	0	1	3	11	18	38	51	62	69	72
Untreated	0	72	0	0	8	28	37	47	58	66	72	
	0	72	0	0	5	11	20	41	51	66	70	71

		t-values		
Untreated vs. Jeffrey	1.0 ml.	2.0	.1	.0
	.1 ml.	.9	.0	.4
Untreated vs. Ponderosa	1.0 ml.	2.1	2.7	3.4
	.1 ml.	3.5	4.8	4.3

t at 90% = 1.81
95% = 2.23

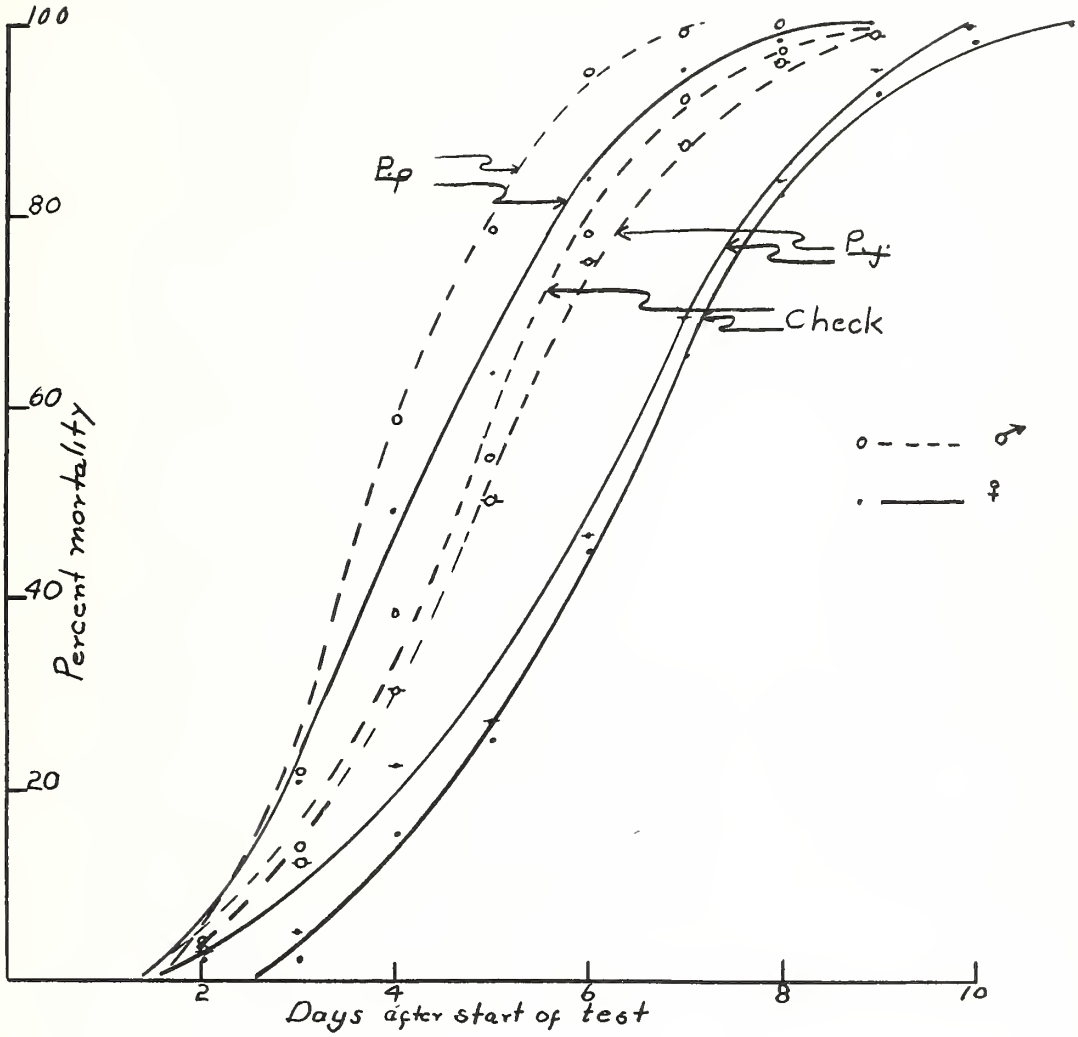

Figure 11.--Effect of sex on mortality rate of D. brevicomis with semi-confined resins of P. ponderosa and P. jeffreyi.

Discussion: Unconfined vapors of Jeffrey resin have no effect on the beetle. Unconfined vapors of ponderosa resin have a significant effect on the beetle. The difference due to sex were parallel to the difference obtained with confined vapors, with the female mortality rate not as rapid as the male.

CONTACT TOXICITY STUDIES

For the same reasons noted under the fumigant studies, gross fresh resin rather than fractionated components was the material selected for testing. The work moved slowly because of the effort directed toward fumigant studies and also because of the difficulties encountered in handling fresh resin. It was immediately evident that the physical instability of resin, primarily evident in the rapid crystallization upon exuding from the tree, would complicate its use in the laboratory. Therefore it was decided to "move the lab to the tree." Even here difficulties were encountered in securing and maintaining the desired flow of resin on the beetle. It was not until September that a procedure was developed which showed some promise. This procedure still was not satisfactory.

The procedure was as follows:

Using a standard increment borer, a hole was made through the bark and $\frac{1}{4}$ inch or more into the sapwood (figure 12). The hole was made on a slightly upward slant to insure a free flow of resin. A hole, 13 mm. in diameter, was punched through the bark and phloem to the sapwood. A cork with a hole 7 mm. in diameter was fitted into this wound. A piece of glass tubing was inserted into the hole in the cork and forced against the increment hole in the sapwood. The glass tubing was 6-inches long and had an 80° bend in it, about 1 inch back from the end which was in the cork. A small piece of 20-mesh plastic screening was placed about 2 inches in from the other end of the glass tubing. Then just before placing the tube into the cork, a beetle was placed in the tube at the end nearest the angle. The tube, therefore, had one end in the cork; a near-right angle directed the tube downward; and the beetle was kept in the tube by a piece of plastic screening.

As resin exuded from the increment hole, it flowed down the tube and came in contact with the beetle. The time was recorded when the beetle first made contact with resin. The time required for the resin to kill the beetle was obtained by constant observation.

Even with this procedure there was a tendency for the resin to crystallize around the beetle. Likewise there was no way of regulating the resin flow so that each beetle encountered the same amount of resin.

The results are given in figure 13.

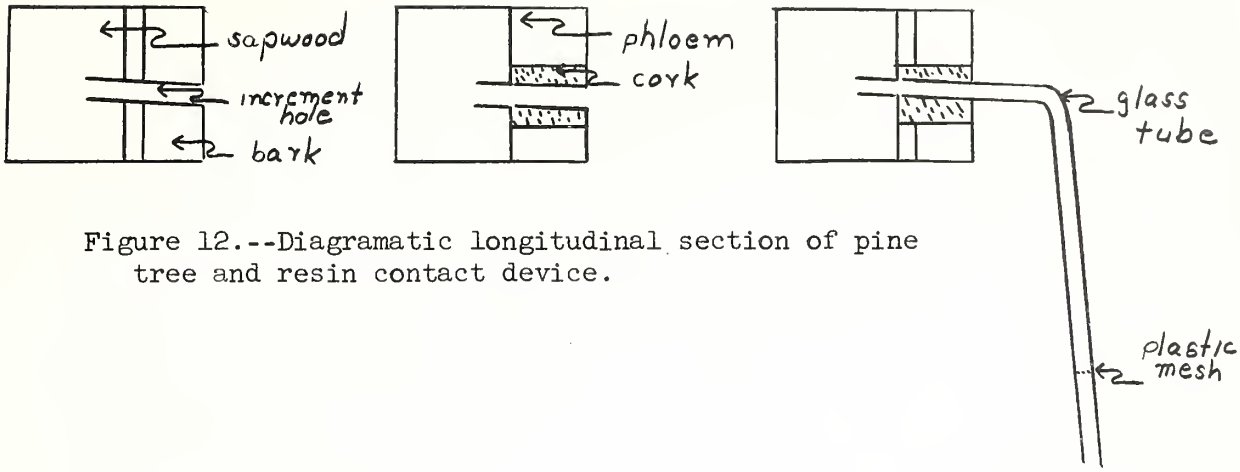

Figure 12.--Diagramatic longitudinal section of pine tree and resin contact device.

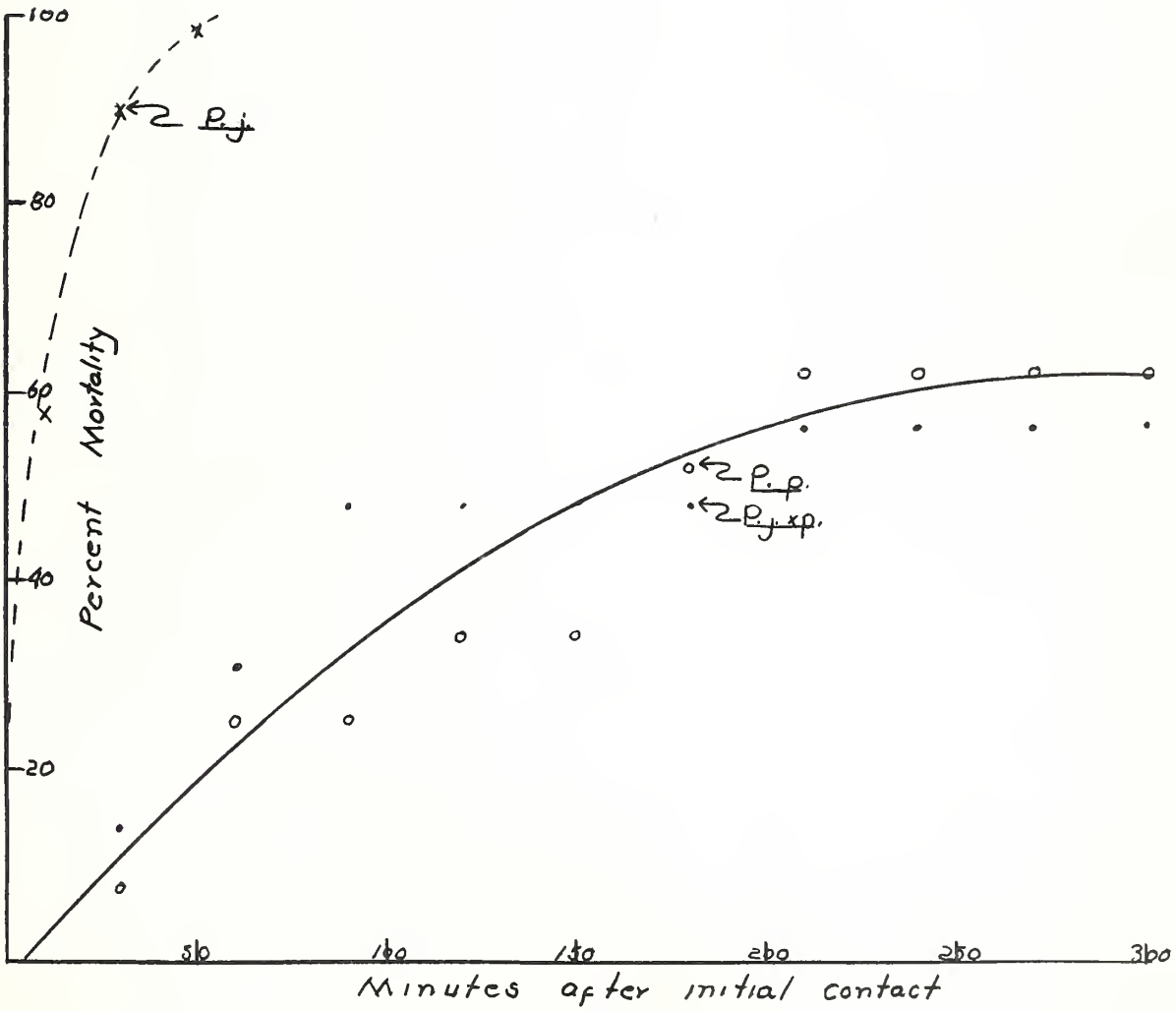

Figure 13.--Mortality rate of D. brevicomis in continuous contact with resin of P. ponderosa, P. jeffreyi, and P. jeffreyi x ponderosa.

It is difficult to reach any sound conclusions because of the small sample and the lack of control of the factors in the test. However the procedure does present certain possibilities for a study of this kind. With a series of holes in one or several trees, a number of beetles may be watched at the same time.

From this one small test, it appears that the resin of the P.j. x p. has no greater adverse effect on D.b. than does the resin of P.p. On the other hand, the P.j. resin has a much greater adverse effect on the beetle.